REMARKS:

Regarding Claim Rejections – 35 USC 112

Regarding rejection 4:

a. the term "characterized in that" is amended as comprising or wherein. The step (c) of claim 1 is amended to have a clear meaning.

b. the claims is amended to use "or". The three methods both can be well used for present invention.

C. The three methods all can use the step (a) method of claim 1 "labeling mRNA from different sources with DNA fragments having different base orders, and mixing the labeled molecules equally to obtain a template for polymerase chain reaction (PCR);" Therefore, the claims 4-6 are clear.

d. the "mixing the label mRNA fragments---," in claim 1 is amended as "mixing the labeled molecules".

e. in claim 5, the term "a sequence specific to gene sources" is amended as "an arbitrary sequence, used to represent the particular gene source".

f. The answer is the same described in c.

g. The claim is amended as "the DNA fragments".

Regarding Claim Rejections – 35 USC 103

Regarding rejection 6.

Amended claims 1-4 and 6-9 overcome the rejection 7 and are patentable under 35 U.S.C. 103(A) over Uematsu et al. (US 6,225,064) in view of Kato (US 6,090,556) and Kaufman et al. (US 6,383,754)as evidenced by Ronaghi et al.," A sequencing method based on real time pyrophosphate".

The currently amended claim 1 is amended to show its new creative features. The amendments are supported by the embodiments 1, 3 and Figs 3, 7.

The differences between the amended claim 1 with Uematsu are as follows: Uematsu's method used dyes with different colors for decoding the source-specific adapters, for example, red color in adaptor I for labeling gene source-I, and green color in adaptor-II for labeling gene source II. While we used different sequences tagged in adaptors for the labeling, for example, sequence "cgat" for gene source-I, and "gcat" for gene source-II. After PCR, the sequence in the amplicon mixture is decoded by pyrosequencing. The merit of our method is the propose of a novel method for dye-free gene expression analysis. Conventionally several dyes are required for labeling the gene source, thus laser is needed for the detection. This determination is expensive and we first invent the dye-free method. Most importantly, the dye's wavelength range only allows the simultaneous comparison of a gene expressed among less than 3 sources, while our method can compare a gene expressed among up to 6 sources at a time. Now pyrosequencing is state in art, and pyrosequencer is already commercialized. But no use of pyrosequencing for gene expression is reported for the moment.

The differences between the amended claim 1 with Kato are that the mRNA for amended claim 1 is labeled by DNA fragments having

different base orders. In Kato the mRNA is labeled by dye. In the amended claim 1 a sequence of amplified DNA mixture means plural amplicons and each represents a gene source as seen in [0013], and Fig. 2. The targets for pyrosequencing are plural species of single-stranded DNAs, each representing a gene source. In a pyrogram (for example, Fig. 5), each peak represents an amplicon corresponding to a gene source as shown in [0031]. One pyrosequencing is enough for comparing a gene expressed in different sources.

However, in the Kaufman's method, the target for pyrosequencing is a single kind of DNA fragment which is amplified from a single DNA species. Conventionally pyrosequencing is carried out on a single template species, and the purpose is to detect the order of the target sequence. Kaufman's method is only the use of pyrosequencing on the template resulting from an adaptor-ligated DNA. The expression level is not obtained by the comparison of peak intensity in a single pyrosequencing, but by plural pyrosequencing on various templates individually. In Kaufman's method, each index sample of binary sequence tags is diluted to achieve a concentration of less than one molecule per well in a microtiter plate before PCR. For detecting one sample, many wells in each of which PCR should be carried out are required, and the expression level of a given binary sequence tag is given by the number of times the sequence occurs. So Kaufman's method is tedious for gene expression analysis.

Therefore, the currently amended claim 1 overcome the rejection 6 and is patentable.

The original claim 2 and currently amended claim 3 are dependent claims of the currently amended claim 1. They hold all new features of the amended claim 1, therefore, they are patentable.

The currently amended claim 4 is dependent claim of the currently amended claim 1, it further defines the claim 1 by that the three methods all use labeling mRNA from different sources with DNA fragments having different base order. The cited references teach nothing about it, therefore, the currently amended claim 4 is patentable.

The currently amended claims 5 and 6 are dependent claims of the currently amended claim 4, they hold all new features of the amended claim 1 and 4, therefore, they are patentable.

The original claim 7 is dependent claim of the currently amended claim 1, it holds all new features of the amended claim 1 and further defines the claim 1 by that the bioluminometric assay is based on a quantitative determination of pyrophosphate released from an extension reaction, which is not disclose by the cited references. Therefore, the original claim 7 is patentable.

The claims 8, 9, 10 are dependent claims of the original claim 7, they hold all new features of the amended claim 1 and original claim 7, therefore, they are patentable.

The new claim 11 is dependent claim of the currently amended claim 1, it holds all new features of the amended claim 1 and further defines the claim 1 by labeling mRNA from six kinds of sources with DNA fragments having six kinds of different base orders, which are not disclosed by the cited references. This was described in the embodiment 3. Therefore, the new claim 11 is patentable.

The new claim 12 is dependent claim of the new claim 7, it holds all new features of the amended claim 1 and new claim 11 and further defines the claim 7 by the six kinds of different base orders include “cgat”, “gcat”, “agct”, “gact”, “cagt” and “acgt”,

only three kinds of dNTPs, including dTTP, dGTP, and dCTP, are used in the bioluminescence analysis, which are not disclosed by the cited references. This was described in the embodiment 3. Therefore, the new claim 11 is patentable.

The rejection 7 is overcome by the amended claims. The reasons are the same described in the remarks for the rejection 6.

For all of the above reasons, applicant submits that the specification and claims are now in proper form, and that the claims all define patentably over the prior art. Therefore, applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

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